U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-269 253

Kepone (Trademark): Chronic Effects on Embryo, Fry, Juvenile, and Adult Sheepshead Minnows ('Cyprinodon variegatus')

Environmental Research Lab, Gulf Breeze, Fla

1977

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing) EPA-600/7-77-044

1 REPORT NO. EPA-600/J-77-031	2 JOURNAL ARTICLE
3 RECIPIENT'S ACCESSION NO. PB 269 933	
4 TITLE AND SUBTITLE: KEPONE: CHRONIC EFFECTS ON EMBRYO, FRY, JUVENILE, AND ADULT SHEEPSHEAD MINNOWS (Cyprinodon variegatus)	5 REPORT DATE
	6 PERFORMING ORGANIZATION CODE
7 AUTHOR(S): David J. Hansen, Larry R. Goodman and Alfred J. Wilson Jr.	8 PERFORMING ORGANIZATION REPORT NO.
9 PERFORMING ORGANIZATION NAME AND ADDRESS: Same as Block 12	10 PROGRAM ELEMENT NO. 1BA613
	11 CONTRACT/GRANT NO.
12 SPONSORING AGENCY NAME AND ADDRESS: Environmental Research Laboratory, Gulf Breeze Office of Research and Development U. S. Environmental Protection Agency Gulf Breeze, Florida 32561	13 TYPE OF REPORT AND PERIOD COVERED
	14 SPONSORING AGENCY CODE EPA/600/04

15 SUPPLEMENTARY NOTES: CONTACT: D.J. Hansen, 8-686-9011 (Gulf Breeze, FL)

REFERENCE: CHESAPEAKE SCIENCE Vol. 18, No. 2, 227-233, June 1977

16 ABSTRACT

We investigated the toxicity of Kepone to, and uptake by embryo, fry, juvenile, and adult sheepshead minnows (Cyprinodon variegatus) using intermittent-flow toxicity tests. Concentrations of Kepone and percentage of adult fish surviving in a 28-day exposure were: Control, 95%; 0.03 µg/liter, 95%; 0.16 µg/liter, 100%; 0.80 µg/liter, 78%; 1.9 µg/liter, 20%; and 7.8 µg/liter and 24 µg/liter, 0%. Concentration factors (concentrations in fish divided by concentrations measured in water) for adult fish averaged 5,200 (range 3,100 to 7,000). Symptoms of poisoning included scoliosis, darkening of the posterior one-third of the body, hemorrhaging near the brain and on the body, edema, fin-rot, uncoordinated swimming, and cessation of feeding. Adults surviving the first exposure were spawned, and the embryonic development, hatching, and survival and growth of fry and juveniles were monitored in a 36-day exposure to Kepone concentrations of 0.08, 0.18, 0.72, 2.0, 6.6, and 33 µg/liter. A significant number of embryos from adult fish exposed to 1.9 µg of Kepone/liter of water developed abnormally and died even when incubated in Kepone-free water. Kepone in water was not as lethal to progeny as to adults: 36-day LC50 for juveniles was 6.7 µg/liter; 28-day LC50 for adults, 1.3 µg/liter. However, the average standard length of juvenile fish was significantly reduced by exposure to 0.08 µg of Kepone/liter of water; some fish developed scoliosis. Concentration factors in juvenile sheepshead minnows averaged 7,200 and increased from 3,600 to 20,000 as exposure concentrations decreased.

17 KEY WORDS AND DOCUMENT ANALYSIS

a DESCRIPTORS	b IDENTIFIERS/OPEN ENDED TERMS	c COSATI Field/Group

REPRODUCED BY NATIONAL TECHNICAL INFORMATION SERVICE U.S. DEPARTMENT OF COMMERCE SPRINGFIELD, VA. 22161

18 DISTRIBUTION STATEMENT: release to public	19 SECURITY CLASS (This Report): unclassified 20 SECURITY CLASS (This page): unclassified	21 NO. OF PAGES 9 22 PRICE PC A02, MF A01

EPA Form 2240-1 (9-73)

EPA-600/3-77-031
JOURNAL ARTICLE

Kepone®: Chronic Effects on Embryo, Fry, Juvenile, and Adult Sheepshead Minnows (*Cyprinodon variegatus*)[1]

ABSTRACT: We investigated the toxicity of Kepone to, and uptake by embryo, fry, juvenile, and adult sheepshead minnows (*Cyprinodon variegatus*) using intermittent-flow toxicity tests. Concentrations of Kepone and percentage of adult fish surviving to a 28-day exposure were: Control, 95%; 0.08 µg/liter, 95%; 0.16 µg/liter, 100%; 0.80 µg/liter, 78%; 1.9 µg/liter, 10%; and 7.8 µg/liter and 24 µg/liter, 0%. Concentration factors (concentrations in fish divided by concentrations measured in water) for adult fish averaged 1,300 (range 3,100 to 7,000). Symptoms of poisoning included scoliosis, darkening of the posterior one-third of the body, hemorrhaging near the brain and on the body, edema, fin-rot, uncoordinated swimming, and cessation of feeding. Adults surviving the first exposure were spawned, and the embryonic development, hatching, and survival and growth of fry and juveniles were monitored in a 36-day exposure to Kepone concentrations of 0.08, 0.16, 0.72, 1.0, 6.8, and 13 µg/liter. A significant number of embryos from adult fish exposed to 1.9 µg of Kepone/liter of water developed abnormally and died even when incubated in Kepone-free water. Kepone in water was not as lethal to progeny as to adults: 36-day LC50 for juveniles was 0.7 µg/liter; 28-day LC50 for adults, 1.3 µg/liter. However, the average standard length of juvenile fish was significantly reduced by exposure to 0.08 µg of Kepone/liter of water; some fish developed scoliosis. Concentration factors in juvenile sheepshead minnows averaged 7,200 and increased from 1,600 to 30,000 as exposure concentrations decreased.

Introduction

Kepone®, decachlorooctahydro-1,3,4-metheno-2H-cyclobuta-cd-pentalen-2-one, has been found recently in estuarine organisms in the James River, Virginia (Hansen et al. 1976). The acute toxicity of this insecticide to estuarine fishes and invertebrates has been investigated (Butler 1963; Schimmel and Wilson 1977), but the chronic effects of Kepone on estuarine fish have not.

This study was conducted to determine: 1) the effects of Kepone exposure on survival and reproduction of adult sheepshead minnows, 2) the effect on survival and growth of embryos, fry, and juvenile fish spawned from exposed adults, and 3) the extent of bioconcentration in adults and progeny.

Materials and Methods

Test Animals

Adult sheepshead minnows were collected near the Environmental Research Laboratory, Gulf Breeze, Florida and acclimated in test aquaria in 30°/oo water for 15 days before exposure. Mortality during acclimation was less than 1 percent, and the fish fed, spawned, and otherwise behaved normally. Tested fish averaged 40 mm standard length and 2.3 g. Adult fish and their food, Biokyst® and frozen adult brine shrimp (*Artemia salina*), contained no detectable (< 0.02 µg/g) Kepone, other chlorinated insecticides or PCB's.

Adult Exposure

We exposed 32 female and 32 male fish in aquaria containing zero, 0.16, 0.80, 1.9, 7.8, and 24 µg/liter of Kepone, and 108 fish to 0.05 µg/liter for four weeks in an intermittent-flow toxicity test. Our apparatus was a modified model of that used by Schimmel et al. (1974). We delivered Kepone, 0.0088 ml of the solvent triethylene glycol, and 1.5 liter of filtered 30°/oo (± 1°/oo) seawater averaging 18°/oo salinity (range 8–26 °/oo) to each 70 liter aquarium during each cycle of the apparatus. Water and solvent without Kepone were delivered to the control aquarium. Number of cycles per day for the adult exposure averaged 440.

Embryo, Fry, and Juvenile Exposure

To determine the effect of Kepone on sheepshead minnow embryo, fry, and juvenile survival, we ex-

® Registered trademark. Allied Chemical Corporation, 40 Rector Street, New York, New York 10006. Kepone used was purchased from Chem Service, West Chester, PA as 99% pure. Our analyses indicated 88% purity. Mention of trade names or commercial products does not constitute endorsement by the Environmental Protection Agency.

[1] Contribution No. 295, Environmental Research Laboratory, Gulf Breeze.

hanced egg production by injecting hormones in exposed adult fish, fertilizing the eggs artificially, and monitoring their development in Kepone for 30 days. Twenty female sheepshead minnows exposed to none, 0.05, 0.16, or 0.50 µg/liter of Kepone and 8 females exposed to 1.9 µg/liter were injected with 30 I. U. of human chorionic gonadotrophic hormone on exposure days 25 and 27 (Hanson et al. 1974). On the 28th day of exposure eggs were stripped manually from injected fish and were fertilized in control water with sperm from excised macerated testes from ten or more males exposed to 0.8 µg/liter or less of Kepone and from five fish exposed to 1.9 µg/liter. Some spawned adult fish from each concentration were frozen for chemical analyses of Kepone content.

Embryos were placed in appropriate aquaria previously used for adult fish to determine the effects of Kepone 1) in water; 2) within the egg; and 3) in both eggs and water on embryo, fry, and juvenile fish. Twenty embryos from control fish were placed in each of four egg cups (Petri dishes to which a nine-cm high collar of 150µ nylon mesh was attached) in the control aquarium and in each aquarium receiving Kepone. Twenty embryos from fish in each aquarium receiving Kepone were placed in each of four egg cups in that aquarium and into four egg cups in the control aquarium. Thus a total of 80 embryos was observed in each of 15 separate treatments. Remaining eggs were frozen for chemical analysis of Kepone content. The drain-fill apparatus cycled about 350 times per day delivering water to each aquarium containing egg cups. The action of a 2/0 starting [illegible] in each aquarium caused water levels to fluctuate approximately 5 cm about 40 times per day insuring that water in the egg cups was exchanged. Fry were fed live brine shrimp nauplii that contained no detectable (<0.02 µg/g) Kepone, other chlorinated insecticides, or PCB's. At the end of the 30-day embryo-fry-juvenile exposure, juveniles were photographed for length measurements, weighed, and some were frozen for chemical analyses.

Chemical Analyses

Water samples were analyzed by extracting one liter of seawater twice with 100 ml of methylene chloride. The combined extracts were concentrated to approximately 5 ml in a Kuderna-Danish Concentrator on a steam table, and 15 ml of benzene was added. The extract was reconcentrated to remove the methylene chloride and was cleaned on a Florisil Column (described below).

Tissues of fish and fish eggs were weighed in 150 mm × 25 mm (0.9 l.) screw-top test tubes and were extracted twice with 5-ml volumes of acetonitrile for 30 seconds with a model PT 10-SI Willems Polytron (Brinkman Instruments, Westbury, New York). The test tube was centrifuged and the acetonitrile was transferred to a 250-ml separatory funnel. After the second extraction, the tissue was extracted with one 5-ml and one 10-ml volume of acetone. After each acetone extraction, the tube was centrifuged and the acetone was added to the 250-ml separatory funnel. To the combined extracts were added 100 ml of 2.0% aqueous sodium sulfate and 10 ml of 1:1 diethyl ether-petroleum ether. The separatory funnel was shaken for one minute. After the solvent phases separated, the lower aqueous phase was drained into a 250-ml beaker and the upper ether layer was collected in a 25-ml Kuderna-Danish concentration tube. The ether extraction was repeated three times with 5 ml of 1:1 diethyl ether-petroleum ether. The combined extracts were concentrated to just dryness by placing the concentrator tube in a water bath at 45°C and blowing off the solvent with a gentle stream of nitrogen. The residue was transferred to a 200 mm × 9 mm (I.D.) chromatoflex column (Kontes Glass Co.) containing 5.0 gm of Florisil topped with 2.0 gm of anhydrous sodium sulfate. The column was washed initially with 10 ml of hexane and the residue was transferred with four 0.5 ml volumes of 6% diethyl ether in hexane. PCB's and pesticides were eluted from the column with 20 ml of 6% diethyl ether in hexane. Then, Kepone was eluted in 40 ml of 1% methanol in benzene. Individual extracts were concentrated or diluted to appropriate volumes for analyses by electron-capture gas chromatography.

Determinations were performed on Varian Aerograph Model 2100 and 1400 Gas Chromatographs equipped with 182 cm × 2 mm (I.D.) glass columns packed with 2% SP2100 and 0.75% SP2250/ 0.97% SP2401 on 100/120 mesh Supelcoport. The operating conditions were: oven temperature, 185°C; injector temperature, 200°C; detector temperature, 210°C; nitrogen carrier gas flow rate, 25 ml/minute.

The average recovery rate of Kepone from fortified tissue was 87% and from water, 85%. Residue concentrations were calculated on a wet-weight basis without a correction factor for percentage recovery. All samples were fortified with an internal standard (dicrotophos-rophenone) prior to analysis to evaluate the integrity of the results.

Statistical Analyses

Probit analyses of mortality data were used to determine LC50's. Chi-square tests ($\alpha = 0.05$) were used to determine statistical significance of mortality data. Analysis of covariance and the Newman-Keuls (SNK) test was used to test differences in growth of fry in Kepone at $\alpha = 0.01$.

Results

Adult Exposures

Kepone was toxic to and was accumulated by adult sheepshead minnows exposed for four weeks (Table 1). Mortality increased in relation to the increase in concentration and duration of exposure (Figs. 1 & 2). All fish exposed to 7.8 and 24 µg of Kepone/liter of saltwater died by day 15. Twenty-two percent of fish in 0.8 µg/liter and 80% of the fish in 1.9 µg/liter died; most of the surviving fish exhibited symptoms of Kepone poisoning.

Symptoms of poisoning were related to concentration and duration of exposure, but were not typical of poisoning by other organochlorine pesticides (Henderson et al. 1959). Symptoms of poisoning progressed from scoliosis, darkening of the posterior one-third of the body, hemorrhaging near the brain and at the anterior point of darkening, to increased hemorrhaging posteriorly, swelling of the darkened area, fin rot, an-

TABLE 1. Toxicity and uptake of Kepone by adult sheepshead minnows (*Cyprinodon variegatus*) exposed for 28 days in an intermittent flow toxicity test. A total of 108 fish were exposed to 0.05 µg/liter Kepone and 32 males and 32 females were exposed to other concentrations. Samples from 1.9 µg/liter consisted of eight females and their eggs and five males; other samples consisted of a minimum of 10 females and their eggs and ten male fish.

Exposure Concentration (µg/liter)			Concentration in Fish (µg/g, Wet Weight)		
Desired	Measured	Mortality, %	Females	Males	Eggs
Control	ND[1]	0	ND[1]	ND	ND
0.1	0.05	0	0.38	0.28	0.26
0.33	0.16	0	0.80	0.65	1.0
1.0	0.80	17	3.6	2.8	3.7
3.2	1.9	50	12	11	11
10.0	7.8	100	–	–	–
32.0	24	100	–	–	–

[1] ND = Kepone not detected: <0.02 µg/liter, <0.02 µg/g.

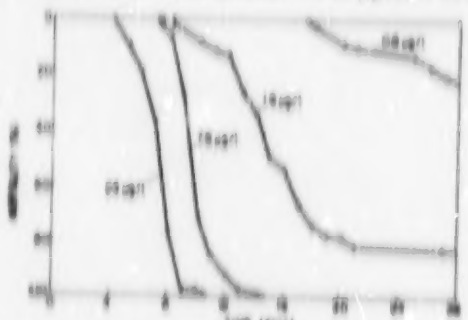

FIG. 1. Mortality of adult sheepshead minnows (*Cyprinodon variegatus*) exposed continuously to Kepone for 28 days.

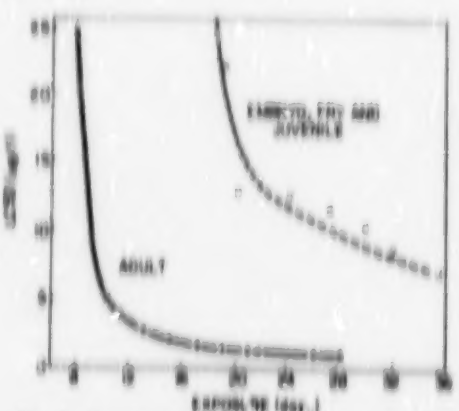

FIG. 2. Concentration of Kepone in water lethal to 50% (LC50) of embryo, fry, and juvenile sheepshead minnows continuously exposed for 36 days and adults exposed for 28 days.

coordinated swimming, and cessation of feeding. These symptoms were observed on the first day in 24 µg/liter, the second in 7.8 µg/liter, the third in 1.9 µg/liter, and the eleventh day in 0.8 µg Kepone/liter of water. These symptoms increased in severity and frequency before death from five to eight days later (Fig. 1).

Chemical analyses of adult sheepshead minnows exposed to Kepone for four weeks showed that Kepone was bioconcentrated in proportion to the concentration in the exposure water (Table 1). Concentration factors, concentration in tissue divided by concentration in water, averaged 5,200 (range 3,100 to 7,000). Concentrations of Kepone in females were greater than in males. Concentrations in eggs were generally similar to amounts in females. The concentration of Kepone in dead fish was similar to that found in fish surviving the exposure (Table 1). Dead fish in 7.8 µg of Kepone/liter of water contained 17 µg/g; in 1.9 µg/liter, 10 µg/g; in 0.8 µg/liter, 3.4 µg/g.

Embryo, Fry, and Juvenile Exposure

Mortality. The effect of Kepone in water on mortality of embryos, fry, and juvenile sheepshead minnows, tested by exposing progeny of adults from the control aquarium to zero, 0.08, 0.16, 0.32, 2.0, 6.6, and 13 µg of Kepone/liter of water, was less than that observed with adult fish exposed to similar concentrations (Table 2, Fig. 2). The 36-day LC50 to juveniles exposed to Kepone in water was 6.7 µg/liter (95% confidence interval: 4.7–13.4 µg/liter) and the 28-day LC50 to adults was 1.3 µg/liter (95% confidence interval: 1.1 to 1.5 µg/liter). Kepone in water affected mortality of embryos prior to hatching. Chi-square tests revealed that mortality of embryos in each Kepone concentration did not differ from that of controls; however, comparisons between embryo mortalities in control, 0.08, and 0.16 µg/liter (average 9%) with concentrations greater than 0.16 µg/liter (average 77%) indicate that embryo mortality was significantly increased by Kepone. Fry from the embryos exposed to 6.6 and 13 µg/liter were also affected within 24 hours of hatching. Juvenile fish exposed to 2.0 or 0.32 µg/liter beginning as embryos did not appear visibly affected until 10 and 16 days after hatching, respectively. Symptoms of poisoning in fry less than one week old included diminished activity, loss of equilibrium, cessation of feeding, and emaciation. Symptoms in fry older than one week were identical to those observed in adult

TABLE 3. Mortality of embryos, fry, and juvenile sheepshead minnows and bioconcentration of Kepone in 19 to 63 juvenile fish (average 50) in a 36-day exposure. In some instances parental fish were exposed to Kepone for 28 days thus their eggs contained Kepone.

Exposure Concentration (µg/liter, measured)		Mortality (%)			
Embryo, Fry, & Juveniles	Parental Fish	Embryos	Fry and Juvenile Fish	Total	Concentration in Juveniles (µg/g, Wet Weight)
Control (ND)[a]	Control (ND)	0.2	3.8	10.0	ND[b]
Control (ND)[a]	0.05	8.8	3.8	12.6	ND
Control (ND)[a]	0.16	5.0	12.5	17.5	ND
Control (ND)[a]	0.80	11.5	0	11.5	ND
Control (ND)[a]	1.9	25.0	3.8	28.5	0.11
0.05	Control (ND)	11.1	11.1	22.2	1.1
0.05	0.05	8.8	0	8.8	1.5
0.16	Control (ND)	8.8	3.8	12.5	1.4
0.16	0.16	6.2	11.2	17.5	1.0
0.72	Control (ND)	21.5	6.3	27.8	2.6
0.73	0.80	13.9	3.8	17.7	1.9
2.0	Control (ND)	15.8	24.4	40.2	2.8
2.0	1.9	26.2	36.3	62.5	6.4
6.6	Control (ND)	17.5	22.5	40.0	22
17	Control (ND)	13.8	86.2	100	-

[a] ND = not detectable, <0.01 µg/l, <0.02 µg/g

fish except for lack of hemorrhaging and the presence of edema in fry exposed to 0.72, 2.0 or 6.6 µg/liter.

Exposure of adult sheepshead minnows to Kepone affected mortality of their embryos in Kepone-free water (Table 3). Sixteen percent of the embryos spawned by adult fish exposed to 1.9 µg/liter failed to develop normally and died; embryos from adults exposed to lesser concentrations developed normally. Fertilization, cleavage, gastrulation, and early differentiation of the embryo including formation of somites, eyes, and circulatory system, appeared normal for about 48 hours. Thereafter, development ceased and although some embryos survived for nine days, all eventually died. Teratogenicity of Kepone might be related to its effect on the gametes or to its presence within the embryo.

In some instances, the presence of Kepone in eggs and water affected fry and juveniles to a greater extent than in water or eggs alone; effect on embryos was not increased (Table 3). Fourteen percent of the embryos spawned by adults exposed to 1.9 µg/liter developed abnormally and died. Mortality of embryos to hatching averaged 7.1% in 0.16 µg/liter or less of Kepone and 20.1% in concentrations greater than 0.16 µg/liter. Fry from adults exposed to 1.9 µg/liter showed symptoms of poisoning one day after hatching at 2.0 µg/liter and as juveniles began to die 10 days later; symptoms were more pronounced and deaths occurred ten days earlier than those for juveniles of unexposed parents. The combined effect of Kepone in eggs and water was negligible at lower concentrations.

Although juveniles that survived 36 days of exposure to 0.05 µg/liter of Kepone showed no symptoms of Kepone poisoning, three of five juvenile fish recovered at termination of the test had scoliosis and blackened tails. These five fish, spawned naturally by adult fish previously exposed to 0.05 µg/liter, were exposed to Kepone longer than were juveniles from the 36-day duration embryo, fry, and juvenile exposure. In Kepone-free water scoliosis persisted for more than 10 days. We believe, therefore, that long-term effects of Kepone on juvenile fish were underestimated in the 36-day test.

Growth. Kepone affected growth of sheepshead minnows (Fig. 3). The average standard length of juveniles exposed to 0.05 to 6.6 µg of Kepone/liter of water was less than that of unexposed juveniles ($P = 0.000001$). Juvenile length decreased in direct proportion to increased concentration of Kepone and was generally unaffected by a history of previous exposure of their parents. Lengths of juveniles in Kepone-free water did not differ even when parents were exposed to Kepone concentrations of 0.05 to 0.8 µg/liter. However, juveniles from parents exposed to 1.9 µg/l were shorter than unexposed juveniles ($p = 0.01$).

Bioconcentration. Chemical analyses of juvenile sheepshead minnows exposed to Kepone in water as embryos and fry for 36 days showed that Kepone was bioconcentrated and that prior exposure of parental fish had little effect on quantities concentrated (Table 3). Concentrations of Kepone in juveniles increased with increased concentrations of Kepone in water. Concentration factors increased from 1,600 to 20,000 (average 7,200) as exposure concentration decreased (Fig. 4). This relationship was not observed with adult sheepshead minnows exposed to Kepone. Juvenile progeny of adult fish exposed to 1.9 µg of Kepone/liter – though hatched and grown in Kepone-free water – contained 0.11 µg Kepone/g or about 36% of the 0.311 micrograms of Kepone originally in an egg remained in the juveniles even after 36 days in Kepone-free saltwater.

Discussion

The hazard of Kepone to fish is greatly underestimated by acute toxicity tests and is incompletely revealed by the results of our tests. Although the 96-hour LC50 of Kepone to sheepshead minnows is 70 µg/liter (Schimmel and Wilson 1977), adult fish in this study

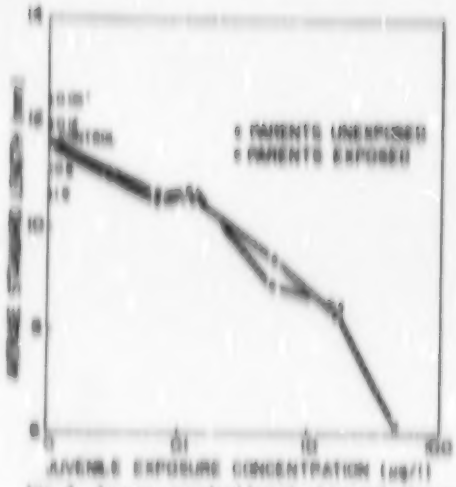

Fig. 3. Average standard length of juvenile sheepshead minnows exposed as embryos and fry for 30 days to zero, 0.08, 0.18, 0.77, 2.6, or 6.6 μg of Kepone/liter of water. Concentrations to which parent fish were exposed were: zero, 0.05, 0.16, 0.80, or 1.9 μg/liter.

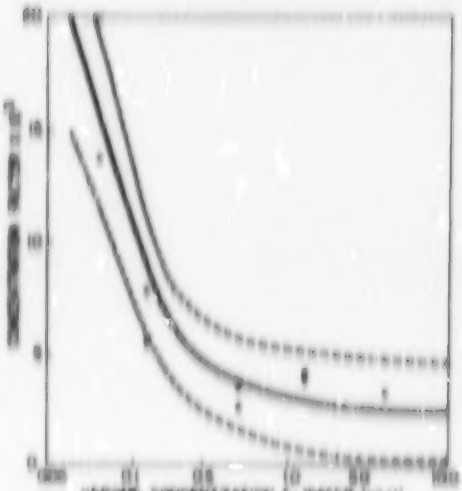

Fig. 4. Concentration factor of Kepone in juvenile sheepshead minnows after 30 days exposure that began with embryos as a function of concentration of exposure. (Concentration factor = concentration measured in juvenile fish divided by concentration measured in water.) Dashed lines indicate 95% confidence limits.

† Concentration of Kepone in water, μg/liter, for parent fish exposed prior to placement of their embryos in Kepone-free water.

died when exposed to concentrations as low as 0.8 μg/liter for 28 days. In exposures that began with embryos, juvenile fish exposed to 0.08 μg/liter were smaller than unexposed control fish. Other effects, such as scoliosis were also observed in these fish. Growth, reproduction, or survival was affected by all concentrations tested. It is generally accepted that 0.01 of the acute toxicity of a persistent organic chemical to an organism should be protective of a species (NAS-NAE 1973). Our data, however, indicate that a Kepone concentration of 0.001 of the 96-hour LC50 for sheepshead minnows affected this species detrimentally in chronic toxicity test.

Reproduction of adult sheepshead minnows was affected by exposure to Kepone in water, but the effect appears to be less pronounced than in birds and mammals fed this insecticide. Some sheepshead minnow embryos from exposed parents developed abnormally, although the majority developed, hatched, and survived as successfully as embryos from unexposed parents. Effects on quail, pheasants, and pigeons have ranged from complete inhibition of reproduction to effects which include cellular, physiological, and endocrinological alterations (De Witt et al. 1961; Elder 1964; McFarland and Lacy 1969; and Tkachenko and Wilson 1975). Kepone in food fed to cows was biomagnified with no apparent ill effect (Smith and Arant 1967), whereas reproduction in mice was reduced as ingested concentrations increased (Good et al. 1965). Our use of a hormone to enhance egg production and artificial spawning procedures may have masked possible additional reproductive effects of Kepone on sheepshead minnows.

Kepone is a highly bioconcentrated pesticide. Bioconcentration factors from 3,600 to 30,000 observed in juvenile sheepshead minnows exposed to Kepone were similar to those observed in juveniles similarly exposed to the insecticides endrin, 3,300–4,800 (Schimmel et al. 1975); chlordane, 8,500–12,300 (Parrish et al. 1976); toxaphene, 6,100–14,400 and heptachlor, 2,400–4,600 (Goodman et al. In press; and to the polychlorinated biphenyls, Aroclor 1016, 2,500–8,000 (Hansen et al. 1975) and Aroclor 1254, 16,000–33,000 (Schimmel et al. 1974). However, the concentration factor for Kepone in juveniles increased with decreased concentration in water. We would expect that concentration factors would have been unaffected by changing concentrations of exposure as observed with juvenile sheepshead minnows exposed to the four insecticides and both PCB's. Concentration factors in adult sheepshead minnows exposed to Kepone, 5,100–7,000; Aroclor 1016, 4,700–14,900 (Hansen et al. 1975); and Aroclor 1254, 15,000–50,000 (Hansen et al. 1974) also were unaffected by change in concentration of exposure.

In these laboratory tests, Kepone was toxic to and accumulated by adult sheepshead minnows and their progeny. This data may be helpful in evaluating the impact of Kepone in estuaries.

Acknowledgments

The significant contributions of Jerrold Forester and Johanie Knight in chemical analyses of water and fish samples and Walter Burgess, Jr. and Charles S. Manning for technical support during the bioassay are gratefully acknowledged.

Literature Cited

Butler, P. A. 1963. Pesticide-Wildlife Studies: A review of Fish and Wildlife Service investigations during 1961 and 1962. U.S. Fish and Wildlife Circ. 167:1–23.

Hewitt, J. B., D. G. Chambers, R. B. Foster, and J. L. Glendon. 1961. Effects on wildlife. In: Effects of pesticides on fish and wildlife in 1960. U.S. Fish and Wildl. Serv. Circ. 143:4–15.

Kenaga, W. H. 1964. Chemical inhibitors of ovulation in the pigeon. *J. Wildl. Mgt.* 28(3):556–575.

Korschgen, V. P., and W. O. Wilson. 1975. Cellular changes in the gonads, livers and adrenal glands of Japanese quail as affected by the insecticide Kepone. *Toxicol. Appl. Pharmacol.* 31: 491–504.

Ohno, E. E., G. W. Ware, and D. F. Miller. 1965. Effects of insecticides on reproduction in the laboratory mouse. I. Kepone. *J. Econ. Entomol.* 58(4):754–757.

Goodman, L. R., D. J. Hansen, J. A. Couch, and J. Forester. 1976. Effects of heptachlor and toxaphene on laboratory-reared embryos and fry of the sheepshead minnow. Proc. 30th Annu. Conf. Southeast. Assoc. Game and Fish Comm., in press.

Hansen, D. J., A. J. Wilson, D. R. Nimmo, S. C. Schimmel, J. H. Haines, and R. Hemmett. 1976. Kepone: Hazard to aquatic organisms. *Science* 193:526.

———, S. C. Schimmel, and J. Forester. 1975. Effects of Aroclor® 1016 on embryos, fry, juveniles, and adults of sheepshead minnows (*Cyprinodon variegatus*). *Trans. Am. Fish. Soc.* 104(3):584–588.

———, S. C. Schimmel, and J. Forester. 1974. Aroclor® 1254 in eggs of sheepshead minnows: Effect on fertilization success and survival of embryos and fry. Proc. 27th Annu. Conf. Southeast. Assoc. Game and Fish Comm., 1973. p. 420–426.

Henderson, C., Q. H. Pickering, and C. M. Tarzwell. 1959. Relative toxicity of ten chlorinated hydrocarbon insecticides to four species of fish. *Trans. Am. Fish. Soc.* 88(1):23–32.

Fabacher, D. L., and P. P. Lucy. 1969. Physiologic and endocrinologic effects of the insecticide Kepone in the Japanese quail. *Toxicol. Appl. Pharmacol.* 15:441–450.

NAS-NAE Committee on Water Quality Criteria. 1973. Water Quality Criteria, 1972. Ecol. Res. Series. 594 pp. U.S. Environmental Protection Agency. EPA-R3-73-033. March 1973. U.S. Govt. Print. Office, Wash., D.C. 20402.

Parrish, P. R., S. C. Schimmel, D. J. Hansen, J. M. Patrick, Jr., and J. Forester. 1976. Chlordane: Effects on several estuarine organisms. *J. Toxicol. Environ. Health* 1:485–494.

Schimmel, S. C., and A. J. Wilson, Jr. 1977. Acute toxicity of Kepone® to four estuarine animals. *Chesapeake Sci.* 18(2):234–237.

———, D. J. Hansen, and J. Forester. 1974. Effects of Aroclor® 1254 on laboratory-reared embryos and fry of sheepshead minnows (*Cyprinodon variegatus*). *Trans. Am. Fish. Soc.* 103(3):582–586.

———, P. R. Parrish, D. J. Hansen, J. M. Patrick, Jr., and J. Forester. 1975. Endrin: Effects on several estuarine organisms. Proc. 28th Annu. Conf. Southeast. Assoc. Game and Fish Comm., 1974. p. 187–194.

Smith, J. C., and F. S. Arant. 1967. Residues of Kepone® in milk from cows receiving treated feed. *J. Econ. Entomol.* 60(4):925–927.

David J. Hansen, Larry R. Goodman and Alfred J. Wilson, Jr.

U.S. Environmental Protection Agency
Environmental Research Laboratory
Gulf Breeze, Florida 32561